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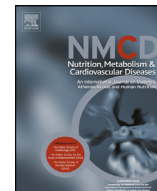
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## Biomarkers and prediction of myocardial triglyceride content in non-diabetic men



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Myocardial  
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**Abstract** *Background and aims:* Lipid oversupply to cardiomyocytes or decreased utilization of lipids leads to cardiac steatosis. We aimed to examine the role of different circulating metabolic biomarkers as predictors of myocardial triglyceride (TG) content in non-diabetic men.

*Methods and results:* Myocardial and hepatic TG contents were measured with 1.5 T magnetic resonance (MR) spectroscopy, and LV function, visceral adipose tissue (VAT), abdominal subcutaneous tissue (SAT), epicardial and pericardial fat by MR imaging in 76 non-diabetic men. Serum concentration of circulating metabolic biomarkers [adiponectin, leptin, adipocyte-fatty acid binding protein 4 (A-FABP 4), resistin, and lipocalin-2] including β-hydroxybutyrate (β-OHB) were measured. Subjects were stratified by tertiles of myocardial TG into low, moderate, and high myocardial TG content groups. Concentrations of β-OHB were lower ( $p = 0.003$ ) and serum levels of A-FABP 4 were higher ( $p < 0.001$ ) in the group with high myocardial TG content compared with the group with low myocardial TG content. β-OHB was negatively correlated with myocardial TG content ( $r = -0.316$ ,  $p = 0.006$ ), whereas A-FABP 4 was not correlated with myocardial TG content ( $r = 0.192$ ,  $p = 0.103$ ). In multivariable analyses β-OHB and plasma glucose levels were the best predictors of myocardial TG content independently of VAT and hepatic TG content. The model explained 58.8% of the variance in myocardial TG content.

*Conclusion:* Our data showed that β-OHB and fasting glucose were the best predictors of myocardial TG content in non-diabetic men. These data suggest that hyperglycemia and alterations in lipid oxidation may be associated with cardiac steatosis in humans.

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**Abbreviations:** A-FABP 4, adipocyte-fatty acid binding protein; β-OHB, β-hydroxybutyrate; CVD, cardiovascular disease; FFA, free fatty acid; HOMA-IR, the insulin-resistance homeostasis model assessment index; LV, left ventricular; MRS, magnetic resonance spectroscopy; T2DM, type 2 diabetes mellitus; TG, triglyceride; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue.

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## Introduction

Intramyocardial triglyceride (TG) content has been reported to be higher in obesity [1], in subjects with the metabolic syndrome [2], impaired glucose tolerance [3], and in type 2 diabetes mellitus (T2DM) [4,5], suggesting that cardiac steatosis is relatively common in humans. Furthermore, increases in intramyocardial TG levels precede the development of cardiac dysfunction, suggesting a causative role of myocardial fat in the development of cardiac dysfunction in these disorders [5,6].

Adipose tissue, apart from its traditional role as energy storage, acts as a source for the production of multiple bioactive molecules including adipokines. These have important roles in the regulation of angiogenesis, blood pressure, glucose homeostasis, lipid metabolism, and vascular haemostasis [7]. The secretion of these molecules is altered in adipose tissue dysfunction that has consequences on vascular health and also cardiac function.

Adipocyte-fatty acid binding protein (A-FABP), leptin, resistin, and lipocalin-2, possess proinflammatory properties, whereas others, such as adiponectin, have an anti-inflammatory effect [8]. A-FABP and adiponectin are the two most abundant adipokines produced by adipocytes. A-FABP 4, a member of FABP family, is a carrier protein that facilitates intracellular FA trafficking from cell membranes to lipid droplets or mitochondria in adipocytes and macrophages. A-FABP 4 has been associated with levels of adiposity, the metabolic syndrome, non-alcoholic fatty liver disease, endothelial dysfunction, atherosclerosis, and cardiovascular disease (CVD) mortality [9].

Limited information exists on the links between adipocyte derived metabolic biomarkers and ectopic fat accumulation in the heart assessed by magnetic resonance spectroscopy (MRS) or magnetic resonance imaging (MRI). Adiponectin has been inversely related with hepatic TG content [10,11], myocardial TG content, epicardial and pericardial fat depots [3,12]. Leptin has been associated with the soleus intramyocellular lipid content [10].

Ketone bodies serve as important energy sources to cardiac and skeletal muscle and nervous tissue during fasting state.  $\beta$ -hydroxybutyrate ( $\beta$ -OHB) belongs to the group of ketone bodies and is formed during FA oxidation by reduction of acetoacetate in the liver. Prolonged exposure to  $\beta$ -OHB has shown to inhibit insulin-stimulated glucose uptake in adult rat cardiomyocytes [13]. In humans, lower basal  $\beta$ -OHB concentrations have been found in obese than in lean individuals [14,15] and in patients with non-alcoholic fatty liver disease [16].

The purpose of the current study was to determine differences in the concentration of different circulating biomarkers potentially modifying lipid and energy homeostasis with increasing amount of intramyocardial TG in a large non-diabetic group of men free of CVD. Our second objective was to examine the association of different biomarkers with different ectopic fat depots. We also evaluated the independent predictors of myocardial TG content.

## Methods

### Study population

A total of 77 men were examined using the same study cohort as have been previously described [2]. A detailed description of the study recruitment can be found in Supplemental Material. Briefly, the subjects were categorized into tertiles based on myocardial TG content: group 1 ( $n = 25$ ) = myocardial TG low, group 2 ( $n = 26$ ) = myocardial TG moderate, and group 3 ( $n = 25$ ) = myocardial TG high. One subject in the group 2 was excluded and considered as outlier due to a highly elevated serum  $\beta$ -OHB concentration. Thirty-seven participants fulfilled the criteria for the metabolic syndrome [17]. In these participants, myocardial ischemia was excluded by means of adenosine stress MR perfusion test. The study was approved by the Helsinki University Central Hospital Ethics Committee and conforms to the principles outlined in the Declaration of Helsinki. Each subject provided written informed consent.

### Demographic variables and biochemical investigations

Waist circumference was determined midway between iliac crest and the lower rib margin. Blood pressure was measured by BPM-200 (Quick Medical, WA, USA) in the sitting position after a 5 min rest, and the mean of five measurements was recorded. The subjects were classified as present, past or non-smokers.

Blood samples were collected after overnight fasting. Total serum cholesterol, TGs, very low-density, high-density lipoprotein cholesterol, FFAs, apolipoprotein B, and high-sensitivity C-reactive protein were analyzed as previously described [2]. The concentration of low-density lipoprotein cholesterol was calculated using the Friedewald formula [18]. Fasting and postload glucose were determined by the hexokinase method (Roche Diagnostic Gluco-quant) using either a Hitachi 917 or a Modular analyzer (Hitachi Ltd, Tokyo, Japan). Serum insulin concentration was assessed by double-antibody radioimmunoassay (Pharmacia RIA kit, Pharmacia, Uppsala, Sweden). The insulin-resistance homeostasis model assessment (HOMA-IR) index was calculated using the following formula: (fasting plasma glucose  $\times$  fasting plasma insulin)/22.5 [19].

We used commercially available enzyme-linked immunosorbent assays for measuring serum levels of adiponectin (R&D Systems, Minneapolis, MN, USA), leptin, resistin (Mediagnost, Reutlingen, Germany), A-FABP 4 (Biovend, Brno, Czech Republic), and lipocalin-2 (R&D Systems), according to the manufacturer's protocol. Serum  $\beta$ -OHB concentration was determined enzymatically (DiaSys Diagnostic Systems, Holzheim, Germany).

### Determination of myocardial and hepatic triglyceride content

Myocardial and hepatic TG content were measured by  $^1\text{H}$ -MRS with a 1.5 T (MAGNETOM Avanto; Siemens AG,

Erlangen, Germany) whole-body MR imager as previously described [2]. Details on measurement of myocardial and hepatic TG content are reported in Supplemental Material.

### Left ventricular analysis and determination of adipose tissue volumes

Cardiac imaging was performed with a 1.5 T whole-body MR imager (MAGNETOM Avanto; Siemens AG, Erlangen, Germany) as previously described [2]. Volumetric analysis of the LV was scrutinized using dedicated post-processing software (Argus, Siemens). LV ejection fraction and mass were reported, and mass was indexed to subject's body surface area and height<sup>2.7</sup>.

The distributions of VAT and subcutaneous adipose tissue (SAT) were determined as previously described [2]. We calculated the VAT/SAT ratio as a metric of abdominal fat distribution [20]. Epicardial and pericardial fat were measured as previously described [2].

### Statistical analyses

All statistical analyses were performed with SPSS 22 for Windows (SPSS, Inc., Chicago, Illinois).

Logarithmic transformations of skewed variables were performed before analysis in order to achieve an approximately normal distribution, when necessary. Data are presented as frequencies or percentages for categorical variables, as means  $\pm$  SD for approximately normally distributed continuous variables, and as medians (range) for skewed variables. Between-group differences for the metabolic biomarkers were examined by one-way analysis of variance and p-values for pairwise between groups difference were adjusted for multiple comparison in a *post hoc* Bonferroni method. Correlations were calculated by the partial correlations coefficients after accounting for age and body mass index. Multivariable regression analyses were used to evaluate the impact of different metabolic biomarkers on myocardial TG content. The final model was decided using the stepwise linear regression procedure,

**Table 1** Clinical and biochemical characteristics, ectopic fat deposits, and left mass and function across tertiles of myocardial triglyceride (TG) content.

	Myocardial TG content low (n = 25)	Myocardial TG content moderate (n = 26)	Myocardial TG content high (n = 25)	P
Myocardial triglyceride content (%)	0.32 (0.14–0.45)	0.74 (0.46–0.87) <sup>b</sup>	1.29 (0.88–2.33) <sup>b</sup>	<0.001 <sup>a</sup>
Age (years)	39 $\pm$ 7	45 $\pm$ 7 <sup>b</sup>	46 $\pm$ 7 <sup>b</sup>	0.032
Body mass index (kg/m <sup>2</sup> )	23.4 (17.6–38.5)	26.6 (22.5–38.6) <sup>b</sup>	30.6 (21.8–42.5) <sup>b</sup>	<0.001 <sup>a</sup>
Waist circumference (cm)	85.5 (71.0–129.0)	97.5 (78.5–125.0) <sup>b</sup>	107.0 (82.5–135.0) <sup>b</sup>	<0.001 <sup>a</sup>
Systolic blood pressure (mmHg)	111 (98–152)	124 (101–153) <sup>b</sup>	128 (99–183) <sup>b</sup>	0.012 <sup>a</sup>
Diastolic blood pressure (mmHg)	73 (61–90)	85 (69–110) <sup>b</sup>	82 (69–119) <sup>b</sup>	<0.001 <sup>a</sup>
Current smokers (N, %)	1 (4)	4 (15)	13 (52) <sup>c</sup>	<0.001 <sup>d</sup>
Total cholesterol (mmol/L)	4.26 $\pm$ 0.85	5.05 $\pm$ 0.79 <sup>b</sup>	5.10 $\pm$ 0.73 <sup>b</sup>	0.001
Very low-density lipoprotein cholesterol (mmol/L)	0.39 (0.07–2.77)	0.70 (0.21–4.65) <sup>b</sup>	1.45 (0.27–3.15) <sup>b</sup>	<0.001 <sup>a</sup>
Low-density lipoprotein cholesterol (mmol/L)	2.42 $\pm$ 0.67	3.14 $\pm$ 0.79 <sup>b</sup>	3.07 $\pm$ 0.66 <sup>b</sup>	0.024
High-density lipoprotein cholesterol (mmol/L)	1.39 $\pm$ 0.41	1.25 $\pm$ 0.33	1.13 $\pm$ 0.42	<0.001
Triglycerides (mmol/L)	0.73 (0.35–3.47)	1.11 (0.55–5.13) <sup>b</sup>	2.20 (0.54–6.26) <sup>b</sup>	<0.001 <sup>a</sup>
Apolipoprotein B (mg/dL)	74 $\pm$ 22	99 $\pm$ 28 <sup>b</sup>	109 $\pm$ 29 <sup>b</sup>	<0.001
High-sensitivity C-reactive protein (mg/L)	0.3 (0.0–9.3)	0.5 (0.0–5.1)	1.6 (0.0–11.8)	0.422 <sup>a</sup>
Fasting free fatty acids ( $\mu$ mol/L)	447 $\pm$ 231	479 $\pm$ 147	517 $\pm$ 178	1.000
Fasting plasma glucose (mmol/L)	5.0 (4.5–6.7)	5.3 (4.4–6.4)	5.8 (4.8–6.9) <sup>b</sup>	0.013 <sup>a</sup>
Fasting serum insulin (mU/L)	2.9 (0.9–22.3)	5.9 (1.5–22.5)	7.8 (1.9–36.9) <sup>b</sup>	<0.001 <sup>a</sup>
HOMA-IR index	0.6 (0.2–6.6)	1.5 (0.3–5.1) <sup>b</sup>	2.0 (0.4–8.0) <sup>b</sup>	<0.001 <sup>a</sup>
Epicardial fat (mm <sup>2</sup> )	494 (283–1666)	695 (373–1753) <sup>b</sup>	838 (295–1666) <sup>b</sup>	<0.001 <sup>a</sup>
Pericardial fat (mm <sup>2</sup> )	497 (118–2649)	1121 (66–6131) <sup>b</sup>	1582 (266–4631) <sup>b</sup>	<0.001 <sup>a</sup>
Hepatic triglyceride content (%)	0.70 (0.17–21.69)	2.37 (0.27–22.01) <sup>b</sup>	6.72 (0.44–31.74) <sup>b</sup>	<0.001 <sup>a</sup>
VAT (cm <sup>3</sup> )	571 (67–5061)	2272 (140–4935) <sup>b</sup>	3304 (350–5743) <sup>b</sup>	<0.001 <sup>a</sup>
SAT (cm <sup>3</sup> )	1443 (284–9188)	2952 (1103–8802) <sup>b</sup>	4663 (871–9354) <sup>b</sup>	<0.001 <sup>a</sup>
VAT/SAT ratio	0.42 (0.14–1.09)	0.60 (0.13–1.55)	0.58 (0.21–1.64)	0.300 <sup>a</sup>
LV ejection fraction (%)	62 $\pm$ 3	61 $\pm$ 5	62 $\pm$ 6	1.000 <sup>a</sup>
LV mass (g)	122 $\pm$ 14	120 $\pm$ 17	134 $\pm$ 22	0.317
LV mass/Body surface are (g/m <sup>2</sup> )	62 $\pm$ 6	57 $\pm$ 9	62 $\pm$ 9	0.500
LV mass/height <sup>2.7</sup>	26 $\pm$ 4	24 $\pm$ 4	27 $\pm$ 4 <sup>c</sup>	0.107

Data are expressed as means ( $\pm$ SD), medians (range) or as frequencies (%). HOMA-IR, the homeostasis model assessment insulin resistance; LV, left ventricular; SAT, subcutaneous adipose tissue; TG, triglyceride; VAT, visceral adipose tissue.

P-values for analysis of variance between myocardial TG content groups, all p-values Bonferroni adjusted for multiple testing.

<sup>a</sup> Analysis of variance based on log-transformed values in order to assure the assumption of normally distributed residuals.

<sup>b</sup> Differs significantly ( $p < 0.05$ ) from myocardial TG content group low. Tests are Bonferroni adjusted for multiple comparisons.

<sup>c</sup> Differs significantly ( $p < 0.05$ ) from myocardial TG content group moderate. Tests are Bonferroni adjusted for multiple comparisons.

<sup>d</sup> P-value based on  $\chi^2$ -test.

**Table 2** Biomarkers across tertiles of myocardial triglyceride (TG) content.

	Myocardial TG content low 0.32 (0.14–0.45) (n = 25)	Myocardial TG content moderate 0.74 (0.46–0.87) (n = 26)	Myocardial TG content high 1.29 (0.88–2.33) (n = 25)	P
S-Adiponectin (ug/mL)	2.54 (0.56–6.83)	1.80 (0.20–5.56)	1.12 (0.59–3.85) <sup>b</sup>	0.125 <sup>a</sup>
S-Leptin (ng/mL)	1.26 (0.01–25.65)	6.38 (1.07–30.54)	8.70 (0.01–30.23)	0.092
S-A-FABP 4 (ng/mL)	7.73 (4.72–21.27)	16.28 (7.48–30.81) <sup>b</sup>	17.22 (7.23–52.33) <sup>b</sup>	<0.001 <sup>a</sup>
S-β-OHB (mg/dL)	1.02 (0.51–4.56)	0.67 (0.46–3.98) <sup>b</sup>	0.68 (0.27–4.89) <sup>b</sup>	0.003 <sup>a</sup>
S-Resistin (ng/mL)	3.03 ± 0.87	3.14 ± 0.86	2.76 ± 0.72	0.200
S-Lipocalin-2 (ng/mL)	60.66 ± 15.88	64.25 ± 13.67	58.02 ± 14.44	0.112 <sup>a</sup>

Data are expressed as means (±SD) or medians (range). A-FABP, adipocyte-fatty acid binding protein; OHB, hydroxybutyrate; TG, triglyceride. P-values for analysis of variance between myocardial TG content groups adjusted for age and body mass index.

<sup>a</sup> Analysis of variance based on log-transformed values in order to assure the assumption of normally distributed residuals.

<sup>b</sup> Differs significantly ( $p < 0.05$ ) from myocardial TG content group low. Tests are Bonferroni adjusted for multiple comparisons.

with age, body mass index, smoking status, and TGs as permanent predictors in the model. The permanent predictors were always included, and not subjects for exclusion during the stepwise procedure. To avoid multicollinearity, parameters of glucose tolerance were not forced into the same model. A  $p$  value  $<0.05$  was considered statistically significant.

## Results

Data on clinical and biochemical characteristics, body composition, and LV mass and function in men with low, moderate, and high myocardial TG content are given in Table 1. Subjects with high or moderate myocardial TG content were older, had higher body mass index, waist circumference, systolic and diastolic blood pressures compared with the group with low myocardial TG content. There were a larger number of current smokers in the high and moderate myocardial TG content groups compared to the low myocardial TG group. The average levels of total cholesterol, low-density lipoprotein cholesterol, TGs, apolipoprotein B, fasting glucose, insulin, and HOMA-IR increased with increasing myocardial TG content. High-

density lipoprotein cholesterol decreased with increasing myocardial TG content [Table 1].

Epicardial and pericardial fat were higher in the groups with high and moderate myocardial TG content compared with the group with low myocardial TG content. In subjects with high myocardial TG content the hepatic TG content (range 0.44–31.74) was ~10-fold higher than in those with low myocardial TG content. Similarly, VAT and SAT were significantly higher in the groups with high myocardial TG content compared with the group with low myocardial TG content. The VAT/SAT ratio was comparable between the study groups [Table 1].

Serum concentrations of biomarkers in the study population divided according to myocardial TG content are presented in Table 2. β-OHB was significantly higher in subjects with low myocardial TG content compared with individuals with high myocardial TG content. A-FABP4 serum levels were higher in the group with high myocardial TG content compared to the group with low myocardial TG content. Leptin levels were higher in subjects with high myocardial TG content compared to subjects with low myocardial TG content but did not reach statistical significance. Concentrations of adiponectin, resistin, and lipocalin-2 were comparable between the study groups (Table 2).

In unadjusted correlation analyses concentrations of β-OHB and adiponectin were negatively correlated with myocardial TG content ( $r = -0.313$ ,  $p = 0.006$  and  $r = -0.480$ ,  $p < 0.001$ , respectively). A-FABP 4 was strongly correlated with myocardial TG content ( $r = 0.607$ ,  $p < 0.001$ ).

Age- and body mass index-adjusted correlation analyses revealed that β-OHB and adiponectin remained negatively associated with myocardial TG content, but A-FABP 4 did not correlate with myocardial TG content. Adiponectin was negatively associated with hepatic TG content, pericardial fat, and VAT. Serum leptin levels were correlated with epicardial fat, VAT, and SAT. A-FABP 4 serum levels were associated with SAT. β-OHB was negatively correlated with VAT. In addition, adiponectin was negatively correlated with TGs. Levels of leptin, A-FABP 4, and resistin were positively correlated with insulin and

**Table 3** Results of stepwise multivariable regression analyses.

Independent variables	Myocardial TG content (log)		
	Unstandardized coefficients		
	β	Std. Error	p
Constant	−8.468	1.759	<0.001
Age (log)	0.765	0.296	0.012
Body mass index (log)	0.493	0.446	0.273
Triglyceride (log)	−0.095	0.117	0.423
fP-glucose (log)	1.711	0.561	0.003
Smoking status	0.258	0.122	0.039
S-Adiponectin (log)	−0.169	0.081	0.042
S-Adipocyte fatty-acid binding protein 4 (log)	0.268	0.140	0.060
B-hydroxy buturate (log)	−0.256	0.080	0.002
Adjusted R <sup>2</sup>		0.588	<0.001

Age, body mass index, smoking status, and triglycerides included as permanent predictors in the model.



**Table 4** Results of stepwise multivariable regression model from Table 3, but with visceral adipose tissue included in the model.

Independent variables	Myocardial TG content (log)		
	Unstandardized coefficients		
	$\beta$	Std. Error	p
Constant	−8.175	1.853	<0.001
Age (log)	0.714	0.313	0.026
Body mass index (log)	0.405	0.477	0.400
Triglyceride (log)	−0.099	0.118	0.404
fP-glucose (log)	1.648	0.576	0.006
Smoking status	0.257	0.123	0.041
S-Adiponectin (log)	−0.161	0.083	0.057
S-Adipocyte fatty-acid binding protein 4 (log)	0.238	0.151	0.120
B-hydroxy buturate (log)	−0.249	0.082	0.003
Visceral adipose tissue (log)	0.050	0.095	0.597
Adjusted R <sup>2</sup>		0.584	<0.001

Age, body mass index, smoking status, and triglycerides included as permanent predictors in the model.

HOMA-IR.  $\beta$ -OHB showed a strong correlation with FFAs (Supplemental Table 1).

Finally, we performed a multivariable regression analysis to further evaluate independent determinants of myocardial TG content. The regression model included all six biomarkers in Table 2 as well as potential confounders (Table 3). This full model had an adjusted explanatory factor of 0.588, i.e. after adjusting for the number of predictors the model explained 58.8% of the variance in myocardial TG content. The model showed age, fasting glucose, smoking status, adiponectin, and  $\beta$ -OHB to be significant independent determinants for myocardial TG content. The model also showed borderline significance for the A-FABP 4. The outcome was closely similar when VAT was included in the full model (Table 4) and when VAT was replaced by hepatic TG content (data not shown). Fasting insulin and HOMA-IR were not significant predictors of myocardial TG content (see Supplemental Tables 2 and 3).

## Discussion

This study showed, in a large cohort of non-diabetic men free of CVD, that  $\beta$ -OHB was significantly lower and A-FABP 4 higher in subjects with high myocardial TG content compared to individuals with low myocardial TG content. In addition,  $\beta$ -OHB was negatively correlated with myocardial TG content, whereas A-FABP 4 was not correlated with myocardial TG content. In multivariable analyses  $\beta$ -OHB and plasma glucose levels were the best predictors of myocardial TG content even beyond VAT and hepatic TG content.

In a normal heart, constant energy supply is primarily met by the  $\beta$ -oxidation of long-chain FAs.  $\beta$ -oxidation accounts for about 50–70% of the energy production of the heart, with the remainder primarily accounted for by carbohydrate (glucose and lactate) oxidation, and the oxidation of ketone bodies [21]. Within the heart, there

normally is little intramyocardial lipid accumulation, suggesting that the uptake and oxidation of FFAs is finely regulated. Overall, the cardiac TG pool is not inert, but rather depends on, and responds dynamically to, the intracellular fluxes of FAs. There are two possible scenarios to explain the accumulation of intramyocardial TG: 1) oversupply of FAs, and 2) impaired FA oxidation. A number of factors, including insulin resistance, contribute to high levels of circulating FAs [22]. Recently another major source of FAs for cardiomyocytes has been reported to be circulating TG-rich lipoproteins hydrolyzed by lipoprotein lipase in the heart [23]. A key regulatory step is the uptake of FFA into cardiomyocytes mediated by FABPs and tissue specific fatty acid transporter proteins, CD36/FAT, and subsequent esterification of FAs. Cardiac insulin resistance is accompanied by a persistent relocation of the fatty acid transporters CD36 and FABP from the cytosol to the cell membrane [22]. Persistent relocation of FA transporters leads to a chronic elevation in FA uptake, which could contribute to the increased FA oxidation observed in diabetic hearts [22]. Genetic studies also indicate a role for proteins involved in FA uptake playing a role in the progression of diabetic cardiomyopathy [22]. We report here that A-FABP 4 serum levels were higher in the group with high myocardial TG content compared to the group with low myocardial TG content. However, A-FABP 4 did not reach significance in the multivariate analyses.

Increasing FA oxidation in the heart downregulates glucose oxidation, while increasing glucose oxidation inhibits FA oxidation. In obese or diabetic heart an elevation of circulating FA is associated with myocardial insulin resistance, and the utilization of excess FA can lead to an impaired consumption of glucose [22]. Furthermore, in the diseased heart glucose uptake rates are elevated, due to hyperinsulinemia and the heart's ability to remain insulin-sensitive [24]. In line with previous reports [2,3] we showed that hyperglycemia was linked to cardiac steatosis. Interestingly, data from recent animal study [25] indicate that hyperglycemia increases myocardial glucose uptake by glucose mass action independently of insulin. Notably, in a recent study including subjects with and without the metabolic syndrome, myocardial steatosis was unrelated to antilipolytic and glucoregulatory actions of insulin [26]. Likewise, our data suggests that insulin resistance is not an independent determinant of cardiac steatosis.

Three compounds are grouped together as ketone bodies: acetoacetate,  $\beta$ -OHB, and acetone. Circulating levels of ketones depend on both the rate of their production by the liver and their use by extrahepatic tissue. The rate of production of  $\beta$ -OHB is dependent on the fluxes of plasma FFA into the liver and is correlated with the rate of lipid oxidation and therefore is considered to be a surrogate marker of lipid oxidation [27]. In the present study, the concentration of  $\beta$ -OHB was unexpectedly significantly lower in subjects with high myocardial TG content but the concentrations of plasma FFA were not reduced indicating disturbed metabolic regulation of ketone bodies.

Similar to FAs, ketone bodies serve as important energy sources to nervous tissue, skeletal and cardiac muscle. Cardiomyocytes oxidize ketone bodies in proportion to their delivery, at the expense of terminal FA oxidation and glucose oxidation as ketone body oxidation is energetically more efficient than terminal FA oxidation [27]. In humans, lower basal  $\beta$ -OHB concentrations have been found in obese than in lean individuals [14,15] and in patients with non-alcoholic fatty liver disease [16]. To our knowledge this has not been previously reported in subjects with excess of myocardial TG content. We also demonstrate an inverse relationship between  $\beta$ -OHB and myocardial TG content. Altogether, our data suggest that myocardial ketone body oxidation may be increased in cardiomyocytes as an adaptive mechanism at the expense of energy shifts between lipid and glucose oxidations in subjects with increased myocardial TG content. Whether this is true remains to be established in positron emission tomography studies in a clinical setting.

This study provided the opportunity to evaluate the relationship between different adipocyte derived metabolic biomarkers and ectopic fat accumulation. As expected, adiponectin levels declined across the study groups, although the difference did not reach statistical significance in age- and body mass index adjusted univariate analysis. In line with previous studies [3,10–12], we observed an inverse relationship between adiponectin and the three fat depots of VAT, myocardial and hepatic TG content. Moreover, in multivariable regression analyses adiponectin was the only independent determinant of myocardial TG content in our biomarker panel. Although adiponectin has numerous beneficial effects on the arterial wall, on the liver, as well as on insulin actions, its independent contribution to the etiology of CVD remains controversial [28].

Strengths of the work are that it was performed in a large cohort of non-diabetic men free of CVD. Six different ectopic fat depots were assessed noninvasively in a quantitative fashion using  $^1\text{H}$ -MRS and MRI. Finally, this study did not consider only one circulating metabolic biomarker, but six biomarkers were simultaneously assessed in an attempt to dissect out their relative importance in predicting myocardial TG content.

On the other hand, we acknowledge some limitations of the study. Both gender and age may influence the magnitude of cardiac steatosis although the data are not consistent [28,29]. Accordingly, only men were included in this study and the data were adjusted to age. However, increased myocardial TG content has also been reported in insulin-resistant obese women [30]. Finally, as the study design is cross-sectional the observational interferences of causality remain limited.

In conclusion,  $\beta$ -OHB is reduced in non-diabetic men with increasing amount of myocardial TG content. In multivariable analyses  $\beta$ -OHB and plasma glucose levels were the best predictor of myocardial TG content even beyond VAT and hepatic TG content. This suggests that reduced lipid oxidation and hyperglycemia may be implicated in the pathogenesis of cardiac steatosis.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.numecd.2015.11.002>.

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## Conflict of interest

The authors report no conflicts of interest.

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